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# ANTIMICROBIAL ACTIVITY AND ANTIBIOTIC SENSITIVITY OF LACTIC ACID BACTERIA ISOLATED FROM FERMENTED FOODS AND A COMMERCIAL PROBIOTIC: A COMPARATIVE IN VITRO STUDY

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***ABSTRACT***

***Five strains of Lactic acid bacteria isolated from fermented foods in the previous study,identified as L. lactis BS14-17, L. lactis YS8-4, L. lactis subsp. cremorisst YS 8-4,Bifidobacterium bifidum NCTC 13001, Bifidobacterium lemurum, labeledas(LAB1, LAB2, LAB3, BIF1,BIF2) & a commercial probiotic Lactococcus lactis (PBIO) were evaluated for their degree of antagonism towards selected human pathogens & food spoilage causing bacteria such as S. aureus, B. subtilis, S. typhimurium, B. cereus, E. coliusing agar well diffusion assay. Three isolates (LAB1, LAB2, LAB3)& the commercial probiotic (PBIO)were studied for their response towards twelve antibiotics by disc diffusion assay. Results were compared by measuring the zones of inhibition for evaluating microbial antagonism & antibiotic sensitivity profiles as well as graphically represented. All five LAB isolates showed microbial antagonism against selected pathogens. Antibacterial activity of LAB isolates varied & ranged between 5-16 mm. On an average LAB isolate BIF1 showed the highest degree of antagonism towards all pathogens. BIF1 showed highest zone of inhibition (16mm) against B. subtilis. Isolates LAB3 & BIF 2 showed the least degree of antagonism.***

***The degree of antibacterial property of isolates & commercial probiotic was in the decreasing order as BIF1 > LAB1> PBIO > LAB2 >BIF2 >LAB3. As such two isolates BIF1 and LAB1 showed more antagonism towards pathogens when compared to commercial probiotic (PBIO). However commercial probiotic showed highestantagonism (ZOI=14mm) towards S. aureus than all LAB isolates in this study. The three isolates of LAB (LAB1, LAB2, LAB3) as well as commercial probiotic (PBIO) were further studied for their antibiotic sensitivity using twelve commercial antibiotic discs. Almost all showed sensitivity towards most of the antibiotics.***

***KEYWORDS: Lactic Acid Bacteria, Microbial Antagonism, Agar Well Diffusion Assay, Antibiotic Sensitivity Probiotics***

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## INTRODUCTION

In recent years interest in the development of new generation of naturally produced antimicrobial agents is on rise due to harmful effects of synthetic chemicals being used as preservatives in food industry. Apart from causing changes in food color or texture, they are reported to cause urticaria, contact dermatitis & some of them even exhibit carcinogenic effects (Kinderlerer & Hatton 1990, John, E.M.,2003).As such consumers demand 'minimally processed'and safe food (Cleveland et al., 2001, Garcia et al., 2016). Moreover, due to intense use of

antimicrobials and antibiotics in foods, pathogenic bacteria have developed resistance to such antibiotics. This has created a global concern in Pharma & food industries (Davidson & Zivanovic 2003, Preidis & Versalovic, 2009).

To develop & isolate antimicrobials from natural sources & to utilize them as bio preservative agents, exploitation of Lactic acid bacteria (LAB) is being considered as an interesting & alternative approach(Settani & Corsetti, 2008.,Todorov SD, et.al 2014, Røssland E, et. al 2005) as they are most beneficial among intestinal microbiota & exist in a variety of food matrices. Interest in exploiting LAB began when famous microbiologist Metchnikoff observed & proved that the health & longevity of Bulgarian peasants was due to consumption of fermented dairy products (Metchnikoff 1908).Lactic acid bacteria has a long history of being used as bio-preservatives in food and feedindustry. The antagonistic character of Lactic acid bacteria (LAB) against pathogenic and spoilage causing micro-organisms was studied and found to be due to production of certain metabolites that exhibit antimicrobial properties such as hydrogen peroxide, Organic acids, diacetyl and bacteriocins (Daeschel, 1989, Holzapfel, et.al, 1995). They target the metabolic functions of other microorganisms, which include protein production, inhibition of ATP and increase in osmotic pressure. In particular, by producing lactic acid these bacteria lower the pH, inhibit the growth and sometimes even kill bacterial pathogens (Vandenbergh, 1993, Amezquita & Brashears, 2002Tambekar&Bhutada, 2010).

Among these LAB metabolites proteinaceousbacteriocins have been studied elaborately especially for their inhibitory role against food borne pathogens in the dairy food preservation (Ruiz-Barbara et.al 1994, Benkerroum et.al 2007). Bacteriocins of LAB are active against strains closely related to the producerstrain. They have gained much attention as natural or so-called ‘bio preservatives’ in food industry because they have been reported to show inhibitory activity against food-borne pathogens such as Listeria monocytogenes, Staphylococcus aureus, Bacillus subtilis and spores of Clostridium perfringens. (Savadogo et. al 2004, Savadogo et. al 2006).As such researchers have focussed on utilizing bacteriocins even for the control of human & animal pathogens.

Extensive research focusing on the exploitation of LAB and their bacteriocins to utilize them in the food industry is due to the fact that vastly done global investigations & research give these bacteria the status of‘generally regarded as safe ( GRAS)& probiotic. (Cleveland et.al 2001, DezGonzalesF. 2007) Also there is no any evidence about the pathogenicity of LAB to humans, (Stiles & Holzapfel, 1997) LABshow strain specificity in exhibiting theirprobiotic potential including antibacterial activity (Eid et.al 2016).

Due to such ideal characteristics, LAB have been considered as the suitable candidates for exploring & utilizing as bio protective agents, due to their antagonistic activity towards target organisms (Triaset. al 2008). As far as the safety assurance of probiotic bacteria to be used in food, FAO/WHO (2002) guidelines emphasize that all probiotic strains be tested for their antibiotic sensitivity- resistance patterns.Also the use of probiotic lactic acid bacteria for gastrointestinal disorders requires their antibiotic resistance profile to enrich the ongoing antibiotic therapy (Salminen et al., 1998).

As such exploring different food matrices for screening LAB with antagonistic characters & studying their antibiotic resistance so as to utilize them in further research is an interesting & optimistic approach. Studies relating to the antibacterial properties of LAB from traditional fermented foods are limited and not fully exploited. Reports showing comparative evaluation of microbial antagonism of LAB & commercial probiotics are rare.

Therefore, this work was carried out with the following objectives.

- Invitro Study of antimicrobial properties of Lactic acid bacteria isolated from fermented foods & a commercial probiotic against common food borne human pathogens& their antibiotic sensitivity profile.
- To compare the exhibited microbial antagonism & antibiotic sensitivity profile of LAB and the commercial probiotic.

## MATERIALS & METHODS

### Bacterial Cultures

Lactic acid bacteria (LAB) used in this study were already isolated by standard microbiological procedures from fermented foods & were selected basing on their folate production ability. Five high folate producers identified asL.lactis BS14-17, L. lactis YS8-4, L.lactis subsp. cremoris YS 8-4,Bifidobacterium bifidum NCTC 13001, Bifidobacterium lemurum,labeled in this study as(LAB1,LAB2,LAB3,BIF1,BIF2)screened & stored as glycerol stocksin MRS broth(15%v/v) at-200C. Commercial probiotic Lactococcus lactislabeled in this study as (PBIO) was purchased in lyophilized form, revived as per standard procedure & used in this study. Pure cultures of selected human pathogens (S. aureus,B.subtilis,S.typhimurium,B.Cereus,E.coli)were obtained from Department of Pathology & Microbiology of a private hospital in Hyderabad India.

### Media

DeMans Rogosa Sharpe broth (MRS broth, Himedia, Mumbai)was used for revival ofstock cultures of LAB, Nutrient broth was used for revival of indicator bacteria & Mueller Hinton agar (MHA) for growth of pathogens in agar well diffusion assay.

Stainless steel cork borer of diameter 5mm was used for boring wells in MH agar. Twelve commercial antibiotic paper discs (Axiom- Multidisc) with known concentrations were used for study of resistance-sensitivity profile of LAB isolates& the commercial probiotic.

### Antibacterial Assay by Agar Well Diffusion Test

In this assay the five isolates of LABscreened as high folate producers (LAB1, LAB2, LAB3, BIF1, BIF2) & a commercial Lactococcus lactis (PBIO) were revived by standard procedures. 5ml of each revived culturebroth was centrifuged at 10,000xg for 10 minutes at $4^0$C in a refrigerated centrifuge. Supernatant was collected and filtered by passing through 0.22micrometer sterile syringe. Thus filtered cellfree supernatant (CFS) was used to study the antagonistic property of LAB against selected pathogens.

Pure cultures of pathogens were revived by inoculating in nutrient broth at $37^0$C for 24 hours. diluted in saline (1% v/v) & this suspension was used for inoculation on Mueller Hinton agar (MHA). For each pathogen two MHA plates were prepared for agar well diffusion assay.

All pathogens were inoculated into MHA plates by spread plate technic using sterile cotton swabs under strict asceptic conditions. Plates were allowed to dry. Three wells in each plate were bored using sterile SS cork borer of diameter 5mm. Each well marked from 1-6 was added with 100 µl cell free suspension of each LAB isolate (No's1-5) & that of commercial probiotic (No.6) obtained in the above step. The added suspension was allowed to diffuse into the agar. Later all the plates were incubated at $37^0$C for 24 hours & observed for zone of inhibition around each well.

The antimicrobial activity was determined by measuring the diameters of all clear zones of inhibition of Lactic acid bacterial isolates &of commercial probiotic around the wells & the results were tabulated.

**Antibiotic Sensitivity Test**

From the above experimentthree isolates (LAB1, LAB2, LAB3) were selected for checking their antibiotic sensitivity. Twelve commercial antibiotic discs (Amikacin, Ampiclox, Ciprofloxacin, Clarithromycin, Cefotaxime, Levofloxacin, Cefuroxime, Cefoperazone, Gentamycin, Roxithromycin, Cotrimoxazole, Azithromycin) with known concentrations were used. MRS medium was prepared, addedin petriplates under aseptic conditions in the laboratory. All three LAB isolates were inoculated by lawn culture technic. Antibiotic Paper discs with known concentrations were placed on the MRS agar plate surface. The plates were kept for incubation aerobically for 24 hours at $37^0$C. The resistance-sensitivity profile of each isolate as well as commercial probiotic were studied by checking the inhibitory zone formed around each antibiotic disc.

## RESULTS

**Antibacterial Test by Agar Well Diffusion Assay**

The agar well diffusion assay was used in this study to compare the antimicrobial activity of five isolated LAB & a commercial probiotic against selected pathogens. The zones of inhibition are shown in the table (1) below. The results are also graphically represented as shown.

**Table 1**

| Pathogen | Zone of Inhibition in mm | | | | | |
|---|---|---|---|---|---|---|
| | LAB1 Isolate | BIF1 Isolate | LAB2 Isolate | BIF2 Isolate | LAB3 Isolate | PBIO (Commercial) |
| S. aureus | **12** | 10 | 9 | 9 | 8 | **14** |
| B. Subtilis | 8 | **16** | 7 | 6 | 7 | 13 |
| S. typhimurium | 9 | 11 | 11 | 8 | 10 | 7 |
| B.cereus | 8 | 7 | 8 | 6 | 6 | **5** |
| L. monocytogenes | 8 | 9 | 8 | 7 | 7 | 8 |
| E.coli | 10 | 12 | 8 | 8 | 6 | 7 |

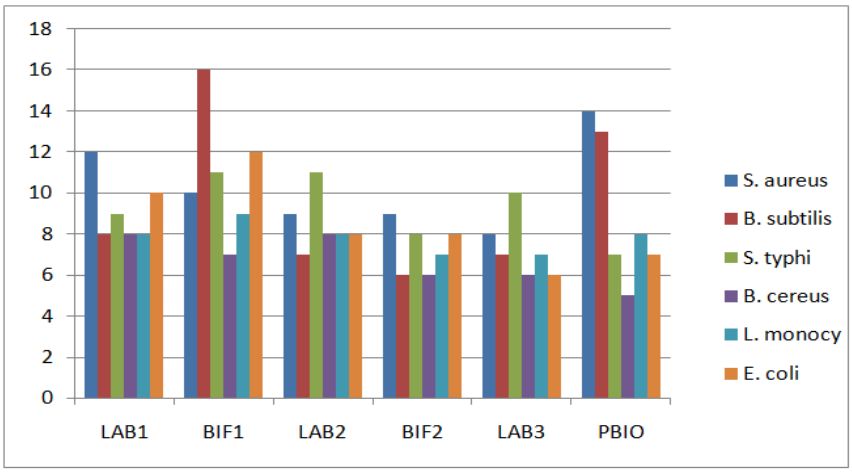


**Figure 1: Graphical Representation of Microbial Antagonism of LAB Isolates Versus a Commercial Probiotic**


**Figure 2: Antimicrobial Activity of LAB Isolates (Zones1-5) &Commercial Probiotic (Zone 6) Against S. Aureus. (Above)**


**Figure 3: Antimicrobial Activity of LAB Isolates (Zones1-5) & Commercial Probiotic(Zone 6) Against B. Subtilis (Above)**

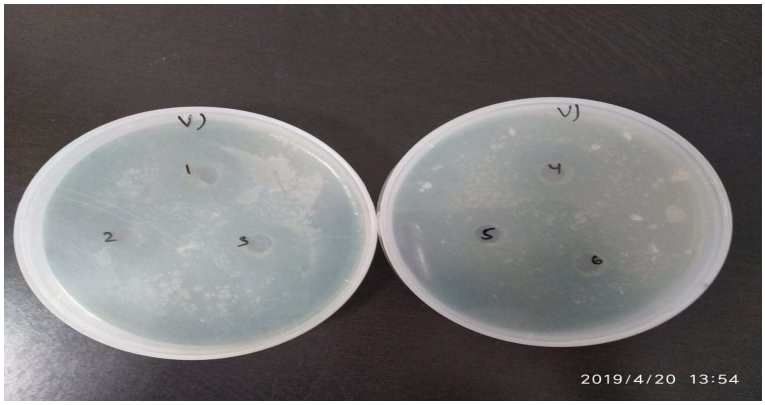


**Figure 4: Antimicrobial Activity of LAB Isolates (Zones1-5) &Commercial Probiotic (Zone 6) Against E.Coli (Above)**

**Antibiotic Sensitivity**

Three Isolates (LAB1, LAB2, LAB3) and commercial probiotic (PBIO) were tested in this study against twelve antibiotics. The results of disc diffusion assay for screening the isolates for their sensitivity toward twelve antibiotics are shown in the table below.

**Table 2**

| Bacteria | Amikacin (AN) | Ampiclox (ACX) | Ciprofloxacin (CIP) | Clarithromycin (CLR) | Cefotaxime (CF) | Levofloxacin (LE) | Cefuroxime (CR) | Cefoperazone CFP) | Gentamycin (G) | Roxithromycin (RX) | Cotrimoxazole (BA) | Azithromycin (AZ) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LAB1 | I | R | S | S | I | S | I | S | S | S | I | S |
| LAB2 | I | I | S | S | R | S | I | S | S | S | S | R |
| LAB3 | I | R | S | S | R | S | S | I | S | S | R | S |
| PBIO (Commercial) | I | R | S | S | I | S | S | I | S | S | I | S |

R= resistance, S= sensitivity, I= intermediate.


**Figure 5: Antibiotic Sensitivity Profile of LAB Isolates (1-3) and Commercial Probiotic (4)**

## DISCUSSIONS

As per the results of antimicrobial activity of LAB isolates of this study All LAB isolates showed bacterial antagonism (antimicrobial property) towards all pathogens studied. The diameters of the zones of inhibition obtained due to inhibitory effect of LAB metabolites present in cell free supernatant of LAB isolates varied in the range between 5-16 mm. Results indicate that the Isolate BIF 1 showed highest zone of inhibition (16mm) against B. subtilis. On an average isolated strain BIF1 showed high degree of antagonism towards all pathogens. Isolate LAB3 & BIF 2 showed least degree of antagonism.

The decreasing order of microbial antagonism of isolates & commercial probiotic wasBIF1 > LAB1> PBIO > LAB2 >BIF2 >LAB3. As such two isolates BIF1 and LAB1 showed more antagonism towards pathogens when compared to commercial probiotic (PBIO). However commercial probiotic showed higher antibacterial property towards S. aureus than all LAB isolates in this study.

The antibiotic sensitivity profile of three isolates LAB1, LAB2, LAB3 and the commercial probiotic Lactococcus lactis (labeled as (PBIO) shows that most of the antibiotics are inhibiting the LAB isolates as well as commercial probiotic Lactococcus lactis strain. LAB1, LAB2 and commercial strain PBIO share the resistance towards Ampiclox antibiotic.

## CONCLUSIONS

Exhibitionof antimicrobial property is one of the important probiotic character of bacteria. In this studysince all five Lactic acid bacterial isolates showed considerable antimicrobial property against selected human pathogens and food spoilage causing bacteria, these isolates can be further studied. Optimum conditions for their growth & metabolism to release antibacterial compounds can be standardized.Their metabolites exhibiting this property can be further investigated by advanced technics such as HPLC, GC to know their category & chemical nature. In this comparative study the isolated Lactic acid bacteria show similarity to commercial probiotic in exhibiting antimicrobial character towards selected pathogens as well as in sensitivitytowards antibiotics. Thus isolated lactic acid bacteria if explored further can be used as commercial probiotics & as bio preservatives in food & feed industry , can be promising alternatives to synthetic chemicals and antibiotics.

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